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Final Report for contract period  
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Study to Determine the Aquatic Biological Effects  
on the Solid Rocket Booster (SRB)

Contract No. NAS 8-32148

by

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# 1. Definition of Problem

During operation of the Space Transportation System (STS), the Solid Rocket Boosters (SRB) will be jettisoned from the Shuttle Orbiter and will parachute to a landing in the sea. After recovery, the SRB will be towed to a disassembly and cleaning facility in the Banana River Estuary at the Kennedy Space Center (KSC), where refurbishment procedures for SRB reuse will be undertaken. During recovery the SRB and parachute recovery system will be immersed in marine waters for several days. Therefore, the surfaces of the SRB will be subject to colonization by marine life, specifically microorganisms. The process of biological colonization of surfaces is generally referred to as marine fouling. The nature and degree of fouling of the SRB material surfaces will have a direct effect on the amount of funds and effort required for cleaning and refurbishment of the SRB, as well as determine the number of times the boosters can be reused. One objective of this study was to develop techniques for monitoring marine microbial fouling of SRB materials to be used in situ in the waters of the SRB recovery zone. The necessary techniques were, in the course of the first year's work, developed and successfully applied to obtain an assessment of the nature and degree of the biofouling of SRB materials to be expected in the ocean and estuarine waters of the recovery zone in the vicinity of the KSC in Florida.

During the second year of research, a series of field studies were conducted to determine the degree of seasonal variations occurring in the levels of microbial fouling in the retrieval zone waters.

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In addition to the SEM technique used in these studies a direct epifluorescence microscopic observation technique for rapid assessment of microbial biofouling of SRB materials was developed. This technique was designed to be used during actual Space Shuttle flights and SRB recovery operations to provide a rapid, real-time method for monitoring SRB microfouling and the effectiveness of SRB cleaning procedures.

The scope of the second year's work was expanded to include the susceptibility of the SRB parachutes recovery system to microbial fouling and biodeterioration. The recovery parachute will be exposed to ocean waters, and damp storage, conditions which allow for microbial biofouling and growth. In addition, the expected 20 re-use episodes for the nylon parachute offers high potential for accumulation of microbial populations and biodeterioration.

Another significant aspect of the research was directed toward determination of the effectiveness of washing techniques in removal of microbial fouling layers from SRB and parachute materials after exposure in the water of the retrieval zone.

## 2. Experimental Approach

### 2.1 Scanning electron microscopy technique

A procedure was devised which permits samples of a variety of SRB material surfaces to be exposed to marine fouling (in the laboratory and in the field), with the same surfaces able to be viewed directly in the scanning electron microscope (SEM) in conjunction with determining the number of microorganisms on the surfaces, using standard microbial isolation techniques on solid (agar) media for direct comparison. Small stubs (0.5 inch diameter surfaces) which fit the

SEM (SEM stubs) were fabricated from two SRB metals, 2219 aluminum and D6AC steel (Fig. 22). The stubs were surfaced with a variety of SRB primers, paints, and sealants, thereby providing miniature samples of SRB material surfaces, simulating exposure of the SRB to marine water and biofouling organisms. The stubs are viewed in the SEM or streaked across solid agar media designed to support growth of marine microorganisms. The SEM observations of the microorganisms attached to the stub surfaces and the results of the enumeration and identification of the organisms, when combined, allow qualitative and quantitative assessment of microbial biofouling of the SRB material surfaces.

## 2.2 Epifluorescence microscopy technique

This technique employs use of small SRB sample surfaces in the form of SEM stubs, exposed in the laboratory and the field, as described in section 2.1. After exposure, the surfaces can be fixed in gluteraldehyde, rinsed, and stained with acridine orange (0.01% solution for 3 minutes). After a final rinse, the surfaces can be viewed under a standard light microscope, adapted for epifluorescence illumination. The acridine orange binds to nucleic acids (DNA and RNA) which are unique compounds found in living organisms. Under ultraviolet illumination, the acridine orange fluoresces (green for DNA, orange for RNA), so that only living organisms are visible in the microscope. The technique uses only reflected ultra violet-induced fluorescent light, and it is, therefore, ideally suited for observation of microorganisms attached to opaque surfaces, such as those of the SRB materials. This technique requires as little as 10 minutes from sample removal from the ocean to observation of

attached microorganisms. Viewing by SEM requires a 1 - 2 day preparation period. In addition, the epifluorescence microscope is portable and could be used on shipboard on a gimbel table, or on shore at the cleaning facility.

3. Field studies of seasonal changes in microbial biofouling of SRB material surfaces in the waters of the SRB retrieval zone

A variety of SRB materials were exposed for various periods of time in the Banana River, at the dockside where the SRB cleaning and refurbishing facility will be located. The sample surfaces, in the form of SEM stubs, were suspended in the river (depth 1 meter), using the tubular sampler developed in the first year of this Research project. Tests were conducted in December 1977 (winter conditions), prior to dredging of the river channel, and in October 1978 (autumn conditions), after dredging of the Banana River. The sample surfaces, after river exposure, were examined by microbiological and SEM techniques. Results were tabulated and compared with data collected previously in March 1977 (spring conditions) and July 1977 (summer conditions), to provide an assessment of seasonal variations in the level of microbial biofouling of the SRB materials.

3.1 Field study in the Banana River in December 1977.

The following SRB materials were exposed in the Banana River for 2 days:

- (1) 2219 Aluminum coated with BOSTIK 463-6-3 primer and BOSTIK epoxy white topcoat 463-3-1. The coating was heated to 306°F, then to 290° at 2 minutes, to 306°F a third minute, air cooled, and irradiated at 375°F.
- (2) D6AC steel coated with Rustoleum 9373 Red Lead-primer and

and Rustoleum 9392 white epoxy topcoat. The coating was heated to 500°F in 5 minutes, 420°F at 3 minutes, 455°F one minute, cooled in air, and irradiated at 610°F.

- (3) 2219 Aluminum, primer and epoxy-coated, surfaced with DC1203 primer and DC-93-076 Dimethylsilicone sealant heated as (1).
- (4) 2219 Aluminum, primer and epoxy-coated, surfaced with PR1422 polysulfide sealant and heated to 275°F, 250°F at 2 minutes, 275°F in third minute, air-cooled, and irradiated at 355°F.
- (5) EH 33-1A nylon parachute material
- (6) Glass (cover slips)

During the test period, the water temperature was ca. 20°C and salinity was 26‰. Microbiological analyses showed that bacteria and fungi attached to the test surfaces after exposure in the river. As in previous tests, the microorganisms, on all test surfaces, were present in numbers too high to be counted directly. These plate cultures were used to provide isolates, after purification identification, for subsequent use in laboratory experiments.

The results of the microbiological analyses, showing microorganisms to be present on all the SRB test surfaces after the 44-hour exposure in the Banana River were confirmed by scanning electron microscopy (SEM). However, the degree of microbial fouling varied with type of material tested, confirming earlier findings for micro-fouling of SRB materials in the Banana River. At 44 hours, the epoxy coated metals, 2219 aluminum and D6AC steel and dimethyl silicone sealant showed similar microbial fouling, including a variety of attached bacteria, as single cells or in discrete microcolonies, as well as attached diatoms (Fig. 1-4). In contrast, the PR1422

polysulfide sealant and woven parachute nylon were far more heavily fouled during the 44-hour exposure period. The polysulfide surface was completely covered with a wide variety of bacteria observed to occur as single cells or in microcolonies (Fig. 5 and 6). In addition, on the polysulfide surface, diatoms and fungi were also evident (Fig. 5 and 6). Filaments appearing to be growing up from the surface suggest that the sealant may be providing nutrients for growth of the microorganisms (Fig. 6).

Thus, even under winter conditions in the Banana River all SRB material surfaces were susceptible to rapid microbial fouling, with the polysulfide sealant showing the greatest susceptibility.

### 3.2 Field study in the Banana River in October, 1978.

The same test materials and procedures described in section 3.1 were used in 46 hour field exposure tests in October 1978 in the Banana River. This test was conducted following completion of dredging of the Banana River Channel. The water temperature during the test period was 25°C and the salinity ranged from 26 to 27‰. Microbiological and SEM analyses of the SRB materials revealed the usual pattern of microfouling susceptibilities. The polysulfide sealant (PR 1422) was most susceptible to microfouling, showing heavy fouling with bacteria and diatoms following exposure for 46 hours in the river (Figs. 7 and 8). In particular, large numbers of filamentous bacteria growing up from the sealant surface were observed (Fig. 7). The painted metal and dimethyl silicone surfaces were also colonized by bacteria and diatoms, but there were areas free of microorganisms, as well (Figs. 9 and 15). The presence of filamentous bacteria attached to and growing up from the surface of the painted

D6AC steel was similar to that observed on the polysulfide (Fig. 10). Another interesting observation was the presence of stalked ciliated protozoans on the painted 2219 aluminum surface (Figs. 14 and 15). The presence of protozoans after only 46 hours exposure is an indication of the rapidity with which fouling occurred during this test. The amount and rapidity with which microfouling occurred on the SRB material surfaces was greater than that observed during cooler seasons of the year, viz. December 1977.

### 3.3 Overall Assessment of seasonal variations in SRB material biofouling.

The range of environmental conditions existing during the seasonal field tests is shown in Table 1. The salinity at the dock-side test site in the Banana River remained relatively high throughout the year, and did not appear to be a significant factor in determining the level of microfouling. Variations in water temperature appear to be significant. The 10°C difference in water temperature, from summer to winter, was reflected in a slight decrease in the amount of microfouling occurring within the test period. Fouling in spring and autumn were similar in rate and extent and were not very much different from that occurring in the summer. Thus, in the Banana River, microbial biofouling will occur rapidly during all seasons of the year on all SRB material surfaces. There was no change noted that would suggest dramatic seasonality in microfouling susceptibility of the various materials. The polysulfide sealant was clearly the most attractive surface for microbial attachment. This observation is in accord with the observed failure of polysulfide sealants due to microbial biodeterioration, communicated

Table 1. Seasonal variation in hydrographic conditions at the dockside test site, Banana River, Florida.

Season	Date	Water Temperature °C	Salinity ‰
Spring	March 1977	26.5	27.5
Summer	July 1977	30.0	32.5
Winter	December 1977	20.0	26.0
Autumn	October 1977	25.5	26.5



to us at the International Biodeterioration Symposium in Berlin in September, 1978 (see appendix).

In the Banana River, sampling of glass (microscope cover glass) surfaced stubs exposed for 44 hours, along with SRB test surfaces was done, with the objective being to introduce a standard surface exposure which ultimately can be employed as an indicator test surface to provide a measure of microfouling occurring during actual Space Shuttle and SRB recovery operations. The glass surface, with respect to microfouling, proved to be comparable to that of the polysulfide surfaces. That is, the glass proved to be an attractive surface for microbial attachment and, thus, may be a useful indicator of fouling to be expected during exposure to sea water. The smooth, clean, corrosion-resistant nature of glass provides an excellent surface for viewing microorganisms. The variety of attached bacteria, singly or in microcolonies, as well as fungal filaments and diatoms can be seen relatively clearly, providing a useful assessment of the kinds and extent of microfouling (Fig. 10 and 11). One of the objectives of this project, therefore, is to establish a standard reference surface for estimating, and eventually quantitating, microbial fouling. At the present time, glass surfaces appear to be appropriate for this purpose, particularly when used as a standard for comparing microbial attachment, using scanning electron microscopy and epifluorescence microscopy.

4. Development of the epifluorescence microscopy technique for direct observation and enumeration of microbial colonization of opaque material surfaces

The approach taken in this part of the research project was to

apply the EMT with laboratory pure cultures under controlled conditions to establish effective staining, and observation techniques and devise statistically interpretable counting procedures. Subsequently, field studies carried out in Chesapeake Bay, Maryland and in the Banana River, Florida, provided a test of EMT developed in the laboratory for natural in situ conditions.

#### 4.1 Laboratory experiments employing the epifluorescence microscopy technique (EMT)

The following preliminary experiment was conducted to test the feasibility of using epifluorescence microscopy to observe and enumerate marine bacteria attached to SEM stub-mounted glass surfaces. Three dilutions of a log phase suspension of a pure culture of the marine bacterium, Vibrio natriegens were made in flasks containing 100 ml of sterile 3 salts nutrient solution (0.25 M NaCl; 3.8 mM KCl; 0.018 M MgSO<sub>4</sub> · 7H<sub>2</sub>O, and 0.01% yeast extract). Each flask contained six alcohol sterilized SEM stubs to which were affixed glass cover-slip surfaces. Cell concentrations at the start of the experiment (determined by spread plate counting) were  $1.5 \times 10^4$  (Flask 1),  $1.5 \times 10^3$  (Flask 2), and  $1.5 \times 10^2$  (Flask 3) cells/ml. Sample stubs were removed from the flasks after exposure for 1 hour, 2 hours and 4 hours to the bacterial suspension and were fixed immediately after removal in a 1.25% glutaraldehyde solution prepared with a buffered 3 salts solution as diluent. The stubs were refrigerated while immersed in the fixative for circa 18 hours, after which the stubs were rinsed with particle-free distilled water (i.e., passed through a 0.2  $\mu$ m filter), stained with a 0.01% acridine orange solution for 3 minutes, rinsed in particle-free distilled

water, and examined under oil immersion, using the epifluorescence microscope. Bacteria observed in 10 randomly selected microscope fields (field area =  $1 \times 10^4 \mu\text{m}^2$ ) were counted for each stub.

The experiment clearly demonstrated that epifluorescence microscopy can be used for direct observation of bacteria attached to opaque surfaces. The data suggest that highest counts i.e., surfaces with the greatest number of attached bacteria, were those in flask 1, which had the highest concentration of cells at the start of the experiment (Table 2). However, in flasks 2 and 3, containing lower cell concentrations, the counts of attached bacteria were too low for statistical significance. In addition, in all flasks observed, there were wide variations in number of attached cells per microscope field, indicating that bacterial attachment on surfaces may not be uniform but is via distribution of clumps, or aggregates. This phenomenon was evident in the 2 hour sample taken from flask 3 (Table 2).

A second set of laboratory experiments were conducted to obtain data appropriate for statistical analysis to distinguish between uniform versus clumped distribution of bacteria attached on surfaces and to assess the validity of the counting procedures for measuring the number of bacteria attaching to surfaces.

Glass surfaces (mounted on SEM stubs) were exposed to bacteria ( $2.0 \times 10^5$  -  $9.2 \times 10^6$  cells/ml) in a low nutrient medium containing 0.01% yeast extract and three salts solutions. Samples were removed and fixed in buffered gluteraldehyde (2.5% G, 0.2M Cacod., in 3 salts) after exposure times of 2, 4, 6, and 22 hours had elapsed. In addition, a 6x stub was removed from the flask containing bacteria exposed for two hours, rinsed with sterile salt solution, and placed

Table 2. Numbers of bacteria attached to glass surfaces after exposure of the surfaces to suspensions of Vibrio natriegens.

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Flask 1 - (initial bacterial concentration  $1.5 \times 10^4$  cells/ml)

<u>Exposure Period</u>	<u>Number of bacteria field<sup>1</sup></u>
1 hour	$9.6 \pm 2.5$
2 hours	$4.3 \pm 1.1$
4 hours	$32.5 \pm 7.8$

Flask 2 - (initial bacterial concentration  $1.5 \times 10^3$  cells/ml)

<u>Exposure Period</u>	<u>Number of bacteria/field<sup>1</sup></u>
1 hour	$0.2 \pm 0.1$
2 hours	$1.2 \pm 0.7$
4 hours	$0.1 \pm 0.1$

Flask 3 - (initial bacterial concentration  $1.5 \times 10^2$  cells/ml)

<u>Exposure Period</u>	<u>Number of bacteria/field<sup>1</sup></u>
1 hour	$0.3 \pm 0.2$
2 hours	$10.1 \pm 4.16^2$
4 hours	$0.2 \pm 0.2$

---

<sup>1</sup> Mean number and standard error of the mean for field area of  $1 \times 10^4 \mu m^2$

<sup>2</sup> Excessively high numbers of cells were observed at the edges of the surfaces, i.e., in the area of the glass where contamination from epoxy cement or handling may have occurred.

in a second flask of sterile salt solution for an additional 4 hours, and fixed. The bacterium used in the experiments is a periphytic (surface attaching) strain isolated from submerged wood surfaces in Puerto Rico. This bacterium was selected for study because it attaches readily to surfaces and is a naturally-occurring, periphytic marine bacterium similar to those encountered in the SRB recovery areas. After fixation for 96 to 144 hours at 4°C the sample surfaces were rinsed in particle-free distilled water, stained with 0.01% acridine orange solution for 3 minutes, rinsed again in distilled water and examined under the epifluorescence microscope.

The bacteria were easily observed and counted using epifluorescence microscopy (See Figure 18). The results of the enumeration of attached bacteria are given in Table 3 and Figure 19. For each time period, two stubs were sampled. Bacteria randomly attached to the surface were counted in 10 selected microscope fields ( $10^{-5} \text{ cm}^2$ ) in each of four quadrants of each stub, i.e. a total of 40 fields/stub and 80 fields for each sampling period were counted. The exception to this procedure was that sample 6X\* comprised only one stub for the counting. Statistical analysis using the F-max test (See Sokal and Rohlf, Biometry, W. H. Freeman Co., San Francisco) of the count data showed no significant differences in counts between quadrants of the same stub, or between duplicate stubs sampled after 2 hour, 4 hour and 6 hour exposure periods. Thus, bacterial distribution over the stub surfaces was uniform at these sampling times. In contrast, statistically significant variation

\* Stub after exposure for two hours to bacteria, was transferred to conditions which did not favor further attachment

(at 95% level) was noted between quadrants of the same stub and between quadrants of the same stub and between duplicate stub samples after 22 hours of exposure to the bacterial suspension, i.e. a more clumped or aggregated distribution (Table 3) was noted. A significant difference in bacterial counts was also recorded for quadrants of the 6x stub.

Statistically significant differences in the mean number of attached bacteria per field were noted between samples exposed for 2 hours and 4 hours and between the 6 hour and 22 hour periods. Differences between 2 hour and the 6X samples and between 4 hour and 6 hour samples were not significant (Figure 19).

Rapid colonization of the glass surfaces was observed, occurring within the first 4 hours, since significant increases in numbers of attached bacteria were recorded between 2 and 4 hours. The attachment rate appeared to level off at 4 and 6 hours, suggesting saturation between 6 to 22 hours, and concluded to be a result of the growth of attached bacteria rather than increased attachment. The hypothesis is offered that attachment is uniform, whereas growth results in clumping, i.e. colony formation. The results of this experiment support such a hypothesis. Thus, in the initial 6 hours, attachment is a dominant factor. The results of the enumeration show no significant internal variation although an increase in variance for the counts was noted with time.

After 22 hours, after surface saturation had occurred, growth was a more important factor. High internal variance in the counts was noted, supported by results for the 6x sample, where the surface, after exposure for two hours to the bacteria, was transferred to conditions whereby attachment was reduced. The resultant increase in bacterial numbers was

accompanied by a significant increase in internal variance for the counts. The increased attachment of the bacteria may be due to growth of cells attached in the initial 2 hours. Experiments to verify the initial observations are underway.

Bacterial colonization of surfaces involves at least two primary processes: (1) attachment of free-living bacteria to a surface, and (2) growth of the bacteria after attachment to the surface. An experiment utilizing epifluorescence microscopy was conducted to determine the contribution of each of the components of the microbial population to the process of microbial colonization of material surfaces.

Glass surfaces mounted on SEM stubs were placed in a large, i.e., 2 liter, flask containing a 3 salts, 0.01% yeast extract medium into which the marine bacterium OWD-1 (at a concentration of  $1 \times 10^6$  cells/ml) had been inoculated. After exposure for two hours in the flask, several stubs were removed and placed into a second, smaller flask (500 ml) containing sterile medium (3 salts, 0.01% yeast extract). The broth in the second flask was circulated through a membrane filter, of 0.22  $\mu$ m pore size, to remove bacteria from the medium. After appropriate times of exposure, sample stubs were removed from each of the flasks, viz. the large flask containing large numbers of bacteria and the smaller flask with low numbers of bacteria. In addition, sterile glass stubs, periodically during the experiment, were added to the large flask and removed after an exposure period of 2 hours. The bacterial concentrations in the flasks were monitored by standard bacteriological methods of dilution and plating on an agar medium.

Sample stubs, after removal from the flasks, were immediately fixed with buffered gluteraldehyde, and stained with acridine orange prior to

examination under the epifluorescence microscope. For each stub, bacteria present in 40 randomly selected microscope fields were counted.

The results of the experiment are presented in Table 2A and Figure 44. In the high concentration flask, i.e., the flask with large numbers of bacteria present, the mean rate of colonization of surfaces based on bacterial counts of the stubs exposed for different 2 hour periods (numbers in parentheses on Table 2A) was 54 bacteria/field/2 hour period. This rate of bacterial colonization of surfaces exposed for the first time remained relatively constant, and appeared to be independent of the number of suspended bacteria in the broth. Due to the high bacterial numbers in the low concentration flask, conditions in that flask were, in fact, a replication of those in the high concentration flask.

Examination of stubs exposed for increasing periods (viz. 2, 4 and 6 hours) revealed nearly identical rate of increase in bacterial numbers on the surfaces exposed in both flasks (Fig. 44). The increases in the number of bacteria on the surfaces between 4 and 6 hours in both flasks at the 54 cell/field rate indicated for the 2 hours exposure periods. Presumably growth (cell division) of bacteria already attached to surfaces was responsible for the higher bacterial numbers observed on those surfaces.

Field studies conducted under this contract have established that bacteria rapidly colonize material surfaces, such as those of the SRB, exposed in marine waters and attain high concentrations on the surfaces within a few hours. The results of the field observations are in accord with results of the experiment reported here. Use of the epifluorescence microscope and the epifluorescent method developed in this laboratory has



been successful. The improved experimental design (viz. better system of bacterial removal and more frequent sampling, after short exposure periods) provides a very promising method for determination of the contribution of bacteria attaching to, and growing on the surfaces of materials in the marine environment.

Table 2A. Mean number of bacteria attached per field on stub surfaces exposed to bacterial suspension and mean number of bacteria in the suspension at the indicated sampling time.

Time	Stubs Flask (high Bacteria)	Bacterial Concentration	Time	<sup>1</sup> Stubs Flask (low Bacteria)	Bacterial Concentration
0	0	$1 \times 10^6$			
0-2H } +2H }	<sup>3</sup> <sub>39-7</sub>	$2 \times 10^6$	0	<sup>2</sup> NO	NO
2-4H	<sup>3</sup> (82-34)				
+4H	<sup>+</sup> <sub>160-39</sub>	$1 \times 10^7$	2	<sup>+</sup> <sub>19-5</sub>	NO
4-6H	<sup>3</sup> (48-6)				
+6H	TNTC	$2 \times 10^7$	4	<sup>+</sup> <sub>64-10</sub>	NO
6-8H	<sup>3</sup> (47-6)				
+8H	TNTC	$4 \times 10^7$	6	<sup>+</sup> <sub>153-12</sub>	$2 \times 10^6$
+12H	TNTC	$6 \times 10^7$	10	NO	NO
+10H	TNTC	$7 \times 10^7$	17	NO	NO

<sup>1</sup> The medium in the flask was filter sterilized on a continuous basis.

<sup>2</sup> Considered to be the same as the 2H sample in the flask (high bacteria).

<sup>3</sup> Bacteria attaching to a sterile stub during the 2 hour time period of exposure.

Table 3. Bacterial colonization of glass surfaces showing results  
of counts and statistical analysis of the count data

Exposure Period	<u>MEAN NUMBER OF BACTERIA/FIELD</u> by quadrants		<u>F Ratios</u> quadrants          stubs	
2 hours	13.6		1.006 ns	1.489 ns
	8.9	14.5		
	17.8			
	17.7			
	13.6			
	10.0	11.7		
	12.9			
	10.3			
4 hours	25.8		1.081 ns	1.296 ns
	23.5	25.9		
	20.7			
	23.5			
	19.1			
	27.7	20.8		
	22.5			
	14.0			
6 hours	23.5		1.762 ns	0.589 ns
	38.2	23.1		
	16.9			
	13.8			
	28.0			
	21.5			
	40.0			
	23.8			
22 hours		<u>x stubs</u>		
	98.9		2.627*	8.602*
	106.7	+ 1002		
	110.5	+ 15.19		
	84.7			
	104.3			
	194.9	+ 166.8		
	200.1	+ 1.196 . 12.69 = 24.87		
	168.1			
<sup>a</sup> 6X	14.3	12.6	3.996*	(N.A.)
	8.4			
	8.1			
	19.5			

\* = statistically significant at the 95 % level

ns = not statistically significant

a = stub exposed to bacterial suspension for 2 hours, then transferred  
to a flask containing sterile medium and incubated for 4 hours.

#### 4.2 Field experiments employing the EMT.

In initial field trials of the method, two surface types, glass and painted 2219 aluminum, were exposed to water in the Rhode River estuary of Chesapeake Bay for periods up to 4 hours. The sample stubs were suspended 1 m below the surface using the tubular sample holder developed in this laboratory and, upon removal, the stubs were immediately fixed with a 2% gluteraldehyde solution made up with a buffered three-salts solution. The water temperature was 19°C and the salinity 9‰ at the sampling site. After standing for 18 hours in fixative at 4°C, the stub samples were rinsed in particle-free distilled water, stained with a 0.01% acridine orange solution for 3 minutes, rinsed a second time and examined under the epifluorescence microscope.

Results of experiments designed to enumerate bacteria attached to glass and painted 2219 aluminum surfaces exposed in the Rhode River for 4 hours are given in Table 4. Counts for surfaces exposed for shorter periods, e.g. 1.5 hours, were too low to be statistically significant and, therefore, are not given. A problem encountered in the enumeration was that the small size of attached bacteria from in situ waters made them more difficult to observe compared with bacteria grown in laboratory culture. This problem did not, however, affect the overall interpretation of the results of the study, i.e., data from the epifluorescence microscopy experiments indicated that glass was more susceptible to microbial attachment than painted 2219 aluminum, a finding consistent with earlier work using scanning electron microscope analyses of microfouling. On both types of surfaces, diatoms occasionally were observed. The diatoms fluoresced under the epifluorescence microscope,

Table 4. Numbers of bacteria attached to material surfaces exposed for 4 hours to Rhode River water in Chesapeake Bay, Maryland.

<u>Fields Counted</u>	<u>Glass Surface</u>	<u>Painted 2219 aluminum surface</u>
1	0	1
2	8	1
3	3	3
4	8	4
5	4	2
6	3	2
7	6	2
8	2	1
9	11	5
10	8	0

Mean number per field and  
standard error of the mean  $5.3^{+1.1}$

$2.1^{+0.5}$

indicating staining with acridine orange. Thus, the diatoms were easily distinguished from bacteria on the material surfaces.

A second field study was conducted in the Rhode River sub-estuary of the Chesapeake Bay on September 21 and 22, 1978. The following test surfaces were suspended in river water, using the tubular sampling system described in previous reports:

1. Glass (cover slip)
2. 2219 aluminum painted with primer and topcoat
3. Painted 2219 aluminum coated with dimethylsilicone sealant (Dow DC93-076)

Sample glass and painted 2219 aluminum surfaces were removed after exposure for 6 and 29 hours in the river. The sealant surfaces were removed after exposure for 29 hours. All samples were immediately fixed in buffered 2.5% gluteraldehyde after removal from the river. Sample surfaces were stained with acridine orange for observation under the epifluorescence microscope, and control samples were dehydrated, critical point dried, and shadowed in preparation for examination under the scanning electron microscope (SEM). Water samples were collected and assayed for bacteria, following standard dilution procedures and colony formation on estuarine salts nutrient agar medium. Hydrographic data were all collected during the course of the field study.

Water temperatures ranges from 25 to 26°C, and the salinity was 10 ‰. The number of heterotrophic bacteria in the Rhode River surface waters ranged from 365 to 650 cells/ml, with a mean value of 510 cells ml.

Attached bacteria were observed on all the test surfaces examined by epifluorescence microscopy. However, enumeration of attached bacteria was made on the glass and painted aluminum surfaces since the

irregularity of the sealant surface prevented statistically accurate counting. The results of the counts using the epifluorescence microscope are given in Table 5. Attached bacteria increased on the glass surface between 6 and 29 hours. Attachment was clearly evident using epifluorescence microscopy, with the mean value after 29 hours of river exposure being significantly (95% level) higher than the mean count after 5 hours. Furthermore, the painted 2219 aluminum surface proved to be a less attractive surface than glass, as evidenced by significantly lower mean values for attached bacteria on the 2219 surface after 29 hours, compared to the glass sample exposed for 29 hours. Interestingly, the mean number of attached bacteria on the glass, after 6 hours, was not significantly different from the mean value for painted aluminum exposed for 29 hours, i.e., a 23 hours longer exposure was needed for fouling of the painted aluminum to the level of the glass after 6 hours, confirming earlier results of our field studies carried out in Florida, whereby glass was found to be a more attractive surface for microfouling than the painted 2219 aluminum surface. SEM examination of duplicate sample surfaces in this Rhode River study yielded results consistent with the epifluorescence microscopy observations.

Epifluorescence microscopy observations of glass surfaces and selected SRB material surfaces exposed in the Banana River for periods up to 46 hours in October 1978 were also made. The glass samples, after fixation, were stained with acridine orange and examined using the epifluorescence microscope. Results of counts of microorganisms attached to glass surfaces, after varied times of exposure in the Banana River are given in Table 6. Bacteria attached to glass surfaces could be observed after exposure for only 2 hours. As exposure time in the Banana River was

Table 5. Number of microorganisms attached to test surfaces. Count (means) are calculated from results of examination of 40 randomly chosen microscope fields.

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<u>Surface</u>	<u>Exposure Period</u>	<u>Mean No. Bacteria per Field</u>	<u>Standard Error of the Mean</u>
Glass	6 hours	10.05	+ 1.15
Glass	6 hours	21.92	+ 2.87
*Painted 2219 aluminum	29 hours	8.60	+ 1.49

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\*The numbers of attached microorganisms on painted 2219 aluminum after 6 hour river exposure were too low for statistical analysis.



increased, the number of microorganisms attached to the surface increased correspondingly. In addition to bacteria, other microorganisms were observed to attach to glass surfaces, but microorganisms other than bacteria were far less numerous and were rarely observed in the microscope fields randomly selected for enumeration. Microorganisms on the SRB material surfaces could be observed readily using epifluorescence microscopy. Background fluorescence of the surfaces, however, made accurate enumeration of the attached flora difficult. Quantitative estimates of attached microorganisms were possible using epifluorescence microscopy techniques on glass surfaces and it is suggested that glass samples be used during actual SRB recovery as an indicator of microfouling potential.

The field studies using the EMT established the utility of this method for observing bacteria and other microorganisms, such as diatoms, that attach to opaque material surfaces. Using the EMT, the number of attached microorganisms can be determined and the numerical data can be interpreted using statistical methods. In addition, differences in rate and extent of attachment onto various types of materials can be quantitated. The data obtained in this study confirmed earlier qualitative assessment of microbial biofouling using SEM procedures.

The EMT is limited to study of flat surfaces which do not give off excessive background fluorescence. Glass provides an ideal study surface in this regard, fitting well with results of SEM studies which established glass as an useful indicator surface for measuring microfouling. The decision to develop a comparative microfouling susceptibility scale using glass as the standard reference surface was thus reinforced.

Table 6. Number of microorganisms attached to glass test surfaces after exposure in the Banana River, Florida. Count (means) given were calculated from examination of 40 randomly selected microscope fields. Results of replicate experiments are given.

Exposure Period	Mean No. Bacteria per field per stub	Standard Error of the Mean	Mean No. Bacteria per field per time period
2 hours	15.02	<u>+2.23</u>	18.89
	22.75	<u>+4.74</u>	
24 hours	132.40	<u>+12.13</u>	91.90
	51.40	<u>+6.18</u>	
46 hours	156.90	<u>+16.09</u>	127.68
	98.46	<u>+ 9.71</u>	

#### 4.3 Overall conclusions concerning the EMT

As a result of this project, EMT has been developed, tested, and found useful in providing an efficient, rapid, direct, and selective method for observing attachment of microorganisms to opaque surfaces. Using glass as a reference surface exposed to microfouling during actual SRB recovery and retrieval operations, the EMT can be used for monitoring microfouling conditions, thereby providing critical information concerning the type of cleaning procedures that will be required.

#### 5. Assessment of microbial biofouling and biodeterioration susceptibility of SRB nylon parachute material

Although exposure of SRB recovery parachutes to the marine environment is shorter than that expected for the SRB, storage under damp conditions and projected 20 reuse episodes increases the likelihood that microbial biofouling and/or biodeterioration will be a significant problem. To assess this potential problem, several field and laboratory experiments were conducted to determine the susceptibility of nylon parachute material to microbial biofouling and biodeterioration.

##### 5.1 Laboratory tests of SRB parachute material.

Parachute nylon samples, mounted on SEM stubs, were suspended in a laboratory aquarium containing artificial seawater, at temperatures ranging from 25 - 27°C, simulating Florida ocean surface water conditions. The aquarium water was inoculated with a mixture of a variety of bacteria and fungi isolated from parachute material exposed in situ to seawater during the Port Everglades field study (see section 5.2 below). Only trace levels of nutrients were added to the aquarium at thirty day intervals, creating conditions favorable to growth of microorganisms capable of degrading the

nylon material. Samples of parachute nylon were removed at established intervals over a period of 8 months for examination by SEM.

SEM observation of parachute nylon exposed to microfouling in the laboratory aquarium revealed that nylon is highly susceptible to microbial biofouling and an increasing pattern of biotic and abiotic deposition on the nylon filaments was observed, with heaviest deposition occurring in the junctions of the woven nylon filaments (Figs. 20-22, 42). These junction areas are considered prime sites for biodegradation. Duplicate samples of the aquarium-exposed parachute nylon were used in washing experiments (see section 6.1).

Although heavy accumulations of material were observed on the aquarium-exposed parachute samples, there was no evidence of gross deterioration of the nylon, viz. pitting or cracking of the filaments. However, decreases in the tensile strength of the parachute nylon can occur. This factor must be assessed in future studies which are planned.

## 5.2 Field tests of SRB parachute material.

Two types of field tests were conducted in December 1977. One involved intermittent exposure (totaling 20 hours) of parachute samples to ocean surface waters and the second consisted of a continuous 44 hour exposure of parachute samples in the Banana River at the dockside test site.

The prototype SRB parachute, with attached parachute samples, was exposed in the Atlantic Ocean off Port Everglades, Florida for five 4-hour periods (total exposure 20 hours). The parachute was then rolled up and stored, while still damp, for 12 days at in situ Florida temperatures.

When the prototype parachute exposed to seawater was unrolled on December 21, 1977 (after storage for 12 days post exposure to seawater),

The sample parachute strips were observed to be damp and the odor emanating from the parachute material indicated that microbial activity was occurring. Samples for microbial and SEM analysis were collected from the following parachute strip samples:

- EH33-3 from parachute base
- EH33-3 from parachute apex
- EH33-8 from parachute base
- EH33-8 from parachute apex
- EH33-8A from parachute base
- EH33-8A from parachute apex
- EH33-4 from parachute base
- EH33-6 from parachute base
- EH33-6A from parachute base
- EH33-10A from parachute base

Results of microbiological analyses indicated that both bacteria and fungi were present on all of the ten parachute samples. Furthermore, the bacteria were too many to be counted. Interestingly, only a few ( 3 ) types of bacteria were present on the surfaces and a similar number of fungal species were isolated. Bacteria and fungi isolated from the parachute samples were purified for identification and use in laboratory studies of biodeterioration. (see section 5.1). Several of the fungal isolates were sent to Ms. Frances Scott at MSFC. These included 3 isolates from the EH33-3 material, 1 isolate from EH33-6 and 2 isolates from the EH8-A sample.

SEM analysis of the sample parachute surfaces confirmed results of earlier microbial analyses. Bacteria were present and growing on the nylon

fibers of all parachute samples examined. Representative micrographs are provided in Figs. 23-28. The presence of bacterial micro-colonies, after intermittent exposure to seawater, confirms earlier findings that nylon parachute material is an attractive surface for attachment of microorganisms. The SEM analysis did not reveal gross biodegradation of the nylon fibers, but clearly established that microorganisms which attack to the parachute during ocean exposure will remain viable during damp storage.

For continuous exposure, parachute samples mounted on SEM stubs were suspended for 44 hours in the Banana River (dockside). The samples were collected, after which they were examined using the SEM. Filamentous fungi were observed on the parachute material, and bacteria were present in very high numbers, completely coating some of the filament surfaces (Figs. 29-31). The bacteria were very similar in morphology, suggesting low diversity, i.e., a selection of bacterial types attaching to the material. Diatoms were not observed on the nylon material within the 44 hour period.

The large amount of bacterial attachment found to occur on the parachute material exposed in the Banana River was in sharp contrast to that of the parachute material that had been exposed intermittently in the Atlantic Ocean off Port Everglades, Florida. The smaller amount of bacterial attachment observed to occur on the parachute material that had been exposed at the open ocean site most likely arises from two factors, the shorter and intermittent ocean exposure periods used (i.e., five 4-hour exposures for a total exposure to open ocean water for 20 hours, as opposed to continuous exposure for 44 hours in the Banana River) and the lower levels of microorganisms found in open ocean water, compared with estuarine (river) waters.

### 5.3 Conclusions regarding microfouling susceptibility of nylon parachute material.

The nylon parachute material is concluded to be highly susceptible to microbial biofouling, since bacterial colonization occurred even with only intermittent exposure to low nutrient ocean waters. Deposits of both biotic (microorganisms) and abiotic (salts) nature can be expected on the nylon material. Gross biodeterioration of the nylon was not observed during these short exposure experiments. However, decreases in tensile strength of the parachute material due to biodeterioration must be tested, in light of the 20 reuse episodes planned for the parachutes. This aspect of our research on the biofouling of parachute material is critical and is strongly recommended for the third year of research under this contract.

### 6. Assessment of the efficacy of washing procedures for removal of biofouling deposits on SRB materials and SRB recovery parachute.

A prime consideration for facilitation of reuse of the SRB and recovery parachute system involves washing the surfaces of these components to remove biotic and abiotic materials deposited during exposure to the marine environment. It is, therefore, essential that an assessment of the effectiveness of washing procedures for removing biofouling deposits be made to determine the resistance of attached microorganisms to removal from material surfaces by washing. Thus, a method was developed, using a commercial laboratory glassware washing machine, whereby the wash consists of two 10-minute spray washes with heated tap water (80°C) and a 10-minute spray wash with heated distilled water (60°C). SEM observations were made on biofouled SRB and parachute materials before and after washing. In one case, soaking with detergent prior to washing was also done.

### 6.1 Washing of SRB materials after field exposure.

Painted 2219 aluminum samples, in the form of SEM stubs, were exposed in the Rhode River, Chesapeake Bay, Maryland, for 29 hours in September 1978. After removal from the river, one set of samples was fixed and examined in the SEM. Observation by SEM revealed that the painted 2219 aluminum surface was colonized by microorganisms, including bacteria, diatoms and several large, stalked protozoans. The protozoans were firmly anchored to the surface by an elaborate holdfast (Figs. 32, 33).

A second set of samples removed from the river were not fixed but, instead, were stored in river water and transported to the laboratory at the University of Maryland. At the laboratory, the unfixed samples were washed in the commercial laboratory glassware washer. After washing, the surfaces were air dried, shadowed with gold-palladium alloy and examined in the SEM. Microorganisms were not discernible on the washed 2219 surface. However, numerous amorphous organic aggregates were present (Figure 34 ). Thus, washing appeared to destroy many of the microorganisms, but an organic layer remained on the surface. In this experiment it was observed that the SEM technique allowed evaluation of the effectiveness of wash procedures. If we are furnished with the washing procedures planned for use on the SRB, the efficacy of the wash procedures for microfouled SRB material surfaces can be determined.

During the seasonal field study in October 1978 SRB materials were exposed to the water of the Banana River, Florida for 46 hours (see section 3.2). SEM observation of polysulfide sealant and painted 2219 aluminum surfaces fixed immediately after the river exposure revealed that considerable microbial biofouling occurred (See Figs. 8, 9, 12-15).



A duplicate set of samples of PR 1422 polysulfide and painted 2219 aluminum was exposed for 46 hours in the Banana River in October 1978. This set was not fixed but instead placed in filter-sterilized Banana River water and transported to the University of Maryland. At the laboratory the fouled sample surfaces were washed in a laboratory washing machine. After washing, the samples were air dried, shadowed with gold-palladium alloy and examined in the scanning electron microscope (SEM).

Examination in the SEM revealed that the standard washing procedure used was not fully effective. Remnants of the microbial fouling organisms were present over the entire surface of the samples. Interestingly, the same relationship observed for microfouling susceptibility of the sample materials was observed after washing, i.e., the polysulfide surface showed greater microfouling residual after washing than the painted 2219 surface (see Figures 35-37). In addition, remains of the more refractory structural components of the microorganisms clearly could be identified after washing. In Figure 37 can be seen the remains of chitin and polysaccharide walls of the mycelium of a fungal filament, and in Figures 35 and 36, the silica-co shells of diatoms attached to the polysulfide surface.

These observations demonstrate that water washes alone will not remove a significant portion of the microbial fouling layer. More elaborate cleaning procedures will have to be employed for effective cleaning of the surfaces of the SRB after ocean retrieval.

#### 6.2 Washing of SRB parachute material following biofouling in a laboratory aquarium.

Nylon parachute samples were exposed in a laboratory aquarium to long-term microbial attack (up to 8 months) by a mixture of microorganisms isolated from the Banana River, under conditions simulating those of the retrieval zone waters (See section 5.1). SEM examination of samples removed

periodically during the 8-month experiment revealed that the nylon surfaces, in particular the junction areas of the woven nylon, developed a heavy deposition of organic and inorganic material. (Figure 42.)

After exposure in the aquarium for 24 hours, three parachute samples were removed; one was fixed and examined by SEM (Figure 38); a second sample was soaked in a 2% sodium lauryl sulfate (detergent) solution for 15 minutes; and the third sample was exposed to the wash cycle in the commercial laboratory washing machine.

From SEM analysis of the washed samples it is concluded that the most effective cleaning procedure was a detergent treatment, which removed nearly all traces of material deposited on the nylon (Figure 40). Water, used alone, was less effective (Figure 39).

Comparison was also made of a parachute sample surface exposed to microfouling for 8 months in the aquarium. Soaking the material in a detergent solution was not included due to a lack of sample material. However, SEM analysis revealed that much of the inorganic material was removed, possibly precipitated salts. However, a significant amount of filamentous organic material remained as a coating on the surfaces of the nylon (Figures 41 and 43). The organic residue can act as a site of microfouling, if the parachute material is reused and, thereby, exposed again to microfouling. There was no apparent gross biodeterioration of the nylon filaments. However, accurate assessment of biodeterioration will require a physical test of the tensile strength of the nylon.

### 6.3 Overall conclusions concerning washing procedures.

The experiments conducted to date clearly demonstrated that water washes are not adequate for cleaning microbial biofouling deposits from

either SRB materials or parachute material, especially for removal of microorganisms, viz., diatoms and fungi, abundant in natural waters and attaching to the surface. Use of a detergent pre-soak provided better cleaning. However, this procedure should be tested on surfaces exposed in the field. In addition, use of detergents will increase the expense of surface cleaning and may damage the SRB materials and parachute surfaces.

7. Summary of conclusions from results of research accomplished to date and recommendations for further study.

A - A method useful for monitoring the rate and extent of biofouling of SRB material surfaces in situ that can be expected to occur in the SRB retrieval and recovery water has been developed, tested and shown to be effective. The method involves a combination of scanning electron microscopy (SEM) and isolation, enumeration, and identification of biofouling microorganisms, using standard microbiological culture methods.

B - A rapid, direct and selective epifluorescence microscopy technique (EMT) for monitoring microbial attachment to material surfaces has been developed and tested. The EMT is effective, providing quantitative, statistically sound evaluation of microbial biofouling of material surfaces. The EMT can be used on shipboard or at the cleaning site, providing data on microfouling within 10 minutes of sample collection. The EMT employs glass as a reference surface and can provide continuous monitoring of microfouling during SRB recovery, retrieval, and cleaning.

C - Biofouling organisms comprise a diverse spectrum of microbial life, including bacteria, fungi, diatoms, protozoans and invertebrate animals. Organic material deposited during biofouling in marine waters

includes a vast array of biochemical macromolecules, which form chemically diverse layers that must be removed during refurbishment of the SRB if the SRB is to be re-used.

D - All SRB materials and the nylon parachute material are susceptible to rapid microbial colonization and when these surfaces are exposed to the marine environment, the materials will be coated with a biologically-derived organic layer of film. The amount of microbial biofouling on the surfaces will be dependent on several factors:

- 1) Length of time of exposure to the marine environment;
- 2) Type of exposure, i.e., biofouling will be greater in the Banana River than in the Atlantic ocean;
- 3) Type of material exposed. The polysulfide sealant and nylon parachute material will be most readily biofouled and most susceptible of the material surfaces examined; and
- 4) Water temperature during exposure. Higher temperatures will result in more rapid biofouling.

E - Some indication of seasonal variation in microbial biofouling was observed, primarily dependent upon water temperature. Biofouling levels were found to be most rapid and extensive during summer months. Thus, the amount of biofouling in the Florida SRB retrieval zone waters will be high throughout the year, with the greatest amount occurring in the summer.

F - Spray washing, with heated tap and distilled water, has been found to be inadequate for removal of biologically derived material deposited on SRB and parachute surfaces after exposure to the marine environment. More effective washing procedures, including use of detergents, must be developed and tested for cleaning biofouling

deposits from the SRB and parachute materials.

G - The parachute material was found to be highly susceptible to microbial biofouling. The biological deposits cannot be removed by water washing. In light of the proposal to reuse the parachutes for 20 missions, each involving repeated exposure to the marine environment, as well as damp storage and drying, the probability is extremely high that the parachutes will deteriorate. Adequate knowledge of the biological and physical effects on parachute tensile strength incurred during repeated use are not yet known and should be determined. Furthermore, an efficient washing procedure for parachute cleaning must be devised and tested. Knowledge of the types of microorganisms capable of causing damage to nylon, e.g. acid-producing fungi, as well as conditions, including temperature and humidity during storage, affecting the biodeterioration must be examined.

Progress on this report has been substantial. The tasks remaining however, are crucial for effective care, maintenance, and proper reuse of the SRB and parachute materials.

## APPENDIX

## Travel Report

Dr. Colwell and Dr. Zachary attended the Fourth International Biodeterioration Symposium at the Bundesanstalt fur Materialprufung (Federal Institute for Material Testing) in Berlin from August 28 through September 1, 1978. Scientists from 23 countries, all of whom were experts working on various aspects of biodeterioration, biodegradation, and biofouling of materials, attended the symposium. A paper entitled "Marine Microbial Colonization of Material Surfaces" by A. Zachary, M.E. Taylor, F.E. Scott and R.R. Colwell was presented at the symposium. The paper described several aspects of the work accomplished with support provided by the NASA contract. The paper was well received by the scientists in attendance and will be published in the Symposium Proceedings. The meeting provided an excellent opportunity to acquaint other scientists with the work being done on this project, to exchange information and ideas about biodeterioration of materials, and to learn of work being done on this subject by internationally recognized experts. During the symposium, new information related to both technical and theoretical aspects of the SRB project was obtained.

Several key papers presented at the symposium concerned the effectiveness of epifluorescence microscopy for monitoring bacterial attachment to surfaces, a method we are developing for use in the Spcae Shuttle Program. In addition, as a result of our presentation, we learned of experiences of other researchers in which polysulfide sealants failed because of severe biodegradation. Thus, our results, showing the high susceptibility of this material to microfouling, observed during field testing, were confirmed.

### Figure Legends

- Figure 1. Bacterial micro-colony showing attachment fibrils among the cells on the surface of epoxy-painted, 2219 aluminum exposed for 44 hours in the Banana River. Magnification 6800X.
- Figure 2. Bacteria (short rods and a long filament) and a pennate diatom attached to an epoxy-painted 2219 aluminum surface that had been exposed for 44 hours at the dockside site in the Banana River in December, 1977. Magnification 4675X.
- Figure 3. Pennate diatom and bacterial micro-colony attached to the surface of epoxy-painted D6AC steel exposed for 44 hours in the Banana River. Magnification 4100X.
- Figure 4. Pennate diatom and bacteria attached to the surface of dimethyl silicone sealant exposed for 44 hours in the Banana River.
- Figure 5. Pennate diatom, fungal filament and bacteria (single cells and micro-colony) attached to polysulfide sealant after exposure for 44 hours in the Banana River. Magnification 3000X.
- Figure 6. Microbial filaments (bacterial and fungal) growing up from the surface of polysulfide sealant after exposure for 44 hours in the Banana River. Magnification 3100X.
- Figure 7. Diatoms and bacteria on the surface of polysulfide sealant after exposure for 46 hours in the Banana River. Magnification 1380X.

Figure 8. Filamentous bacteria growing up from the surface of polysulfide sealant exposed in the Banana River for 46 hours. Magnification 1695X.

Figure 9. Bacteria on painted D6AC steel surface after exposure for 46 hours in the Banana River. Magnification 5000X.

Figure 10. Filamentous microorganisms growing up from painted D6AC steel surface after exposure for 46 hours in the Banana River. Magnification 6700X.

Figure 11. Diatom and bacteria on dimethylsilicone sealant exposed to Banana River water for 46 hours. Magnification 2000X.

Figure 12. Colonial diatom and fungal mycelium on surface of painted 2219 aluminum after exposure for 46 hours to Banana River water. Magnification 112X.

Figure 13. Diatoms, mycelial strands, and bacteria on painted 2219 aluminum after exposure for 46 hours to water in the Banana River. Magnification 1168X.

Figure 14. Chain of diatoms, mycelial strands, protozoans, and bacteria on painted 2219 aluminum after exposure to Banana River water for 46 hours. Magnification 520X.

Figure 15. High magnification of area outlined in Figure 14 showing details of stalked, ciliated protozoan microorganisms. Magnification 4635X.

Figure 16. Fungal filaments, pennate diatom and bacteria on the surface of glass exposed for 44 hours in the Banana River. Magnification 1100X.



Figure 17. Fungal filament, diatoms (Cocconeis) and bacterial microcolonies attached to glass exposed for 44 hours in the Banana River. Magnification 2500X.

Figure 18. Marine bacteria (OWD-1), attached to glass surface after 22 hour exposure. Magnification 1250X.

Figure 19. Numbers of bacteria attached to a glass surface after exposure, under laboratory conditions, for time intervals up to 22 hours. Data are presented as mean number of attached bacteria per field, with 95% confidence intervals.

Figure 20. Parachute nylon subjected to microfouling by microorganisms in a laboratory aquarium. Time of exposure was 8 days. Magnification 190X.

Figure 21. Parachute nylon subjected to microfouling by microorganisms in a laboratory aquarium. Time of exposure was for 33 days. Magnification 111X.

Figure 22. Nylon parachute material exposed to fouling in laboratory aquarium for 50 days. Magnification 67X.

#### Figures

23 - 25. Parachute sample EH-6A from base of prototype parachute after intermittent ocean exposure for 20 hours and damp storage for 12 days. From Figure 23 can be seen the parachute fibers at 275X magnification; Figures 24 and 25 provide greater magnification (430X and 8800X) of the area outlined in Figure 23. The presence of a bacterial microcolony and attachment fibrils are seen in Figure 25.

## Figures

- 26 & 27. Parachute sample EH33-8 from the apex of the prototype parachute after intermittent ocean exposure for 20 hours and damp storage for 12 days. Figure 26 shows the nylon fibers at 700X and in Figure 27 (7000X) the bacterial micro-colony and associated attachment fibrils can be seen in the area outlined in Figure 25.
- Figure 28. Low magnification (280X) of parachute sample EH33-3 from base of prototype parachute after intermittent ocean exposure for 20 hours and damp storage for 12 days.
- Figure 29. Fungal filaments on SRB parachute material (EH33-1A) after exposure for 44 hours in the Banana River. Magnification 290X.
- Figure 30. Bacteria attached to nylon filament of parachute material (EH33-1A) exposed for 44 hours in the Banana River. Magnification 3000X.
- Figure 31. Attachment fibrils binding bacteria to nylon filament of parachute material (EH33-1A) exposed for 44 hours in the Banana River. Magnification 15000X.
- Figure 32. Microorganisms attached to painted 2219 aluminum surface after 29 hour exposure in the Rhode River. Two large stalked protozoa organisms are seen. Magnification 460X.
- Figure 33. Higher magnification of protozoans, showing holdfast structure firmly anchoring the organism to the surface. A small diatom and fine fibrillar network are also present. Magnification 1955X.
- Figure 34. SEM micrograph of painted 2219 aluminum surface after 29 hour exposure to Rhode River water and washing in commercial laboratory

washing machine without detergent. Clumps of amorphous organic material are evident. Magnification 570X.

Figure 35. Residual organic debris on polysulfide sealant surface washed after exposure for 46 hours in the Banana River, (a) surface at 520X magnification (b) fragment of a diatom shell (area in box) at 2600X magnification.

Figure 36. Residual organic debris on polysulfide sealant material washed after exposure for 46 hours in the Banana River, (a) surface at 370X magnification (b) area in white box at 1850X magnification, showing detail of fragment of a diatom shell.

Figure 37. Surface of painted 2219 aluminum washed after exposure for 46 hours in the Banana River, (a) residual organic debris and fungal mycelial remains. Magnification 520X (b) area in white box showing detail of fungal mycelial wall. Magnification 2600X.

Figure 38. Parachute nylon (EH33-3) after exposure to microbial biofouling for 24 hours in a laboratory aquarium. Magnification 190X.

Figure 39. Parachute nylon (EH33-3) after exposure to microfouling for 24 hours followed by tap and distilled water washes. Magnification 190X.

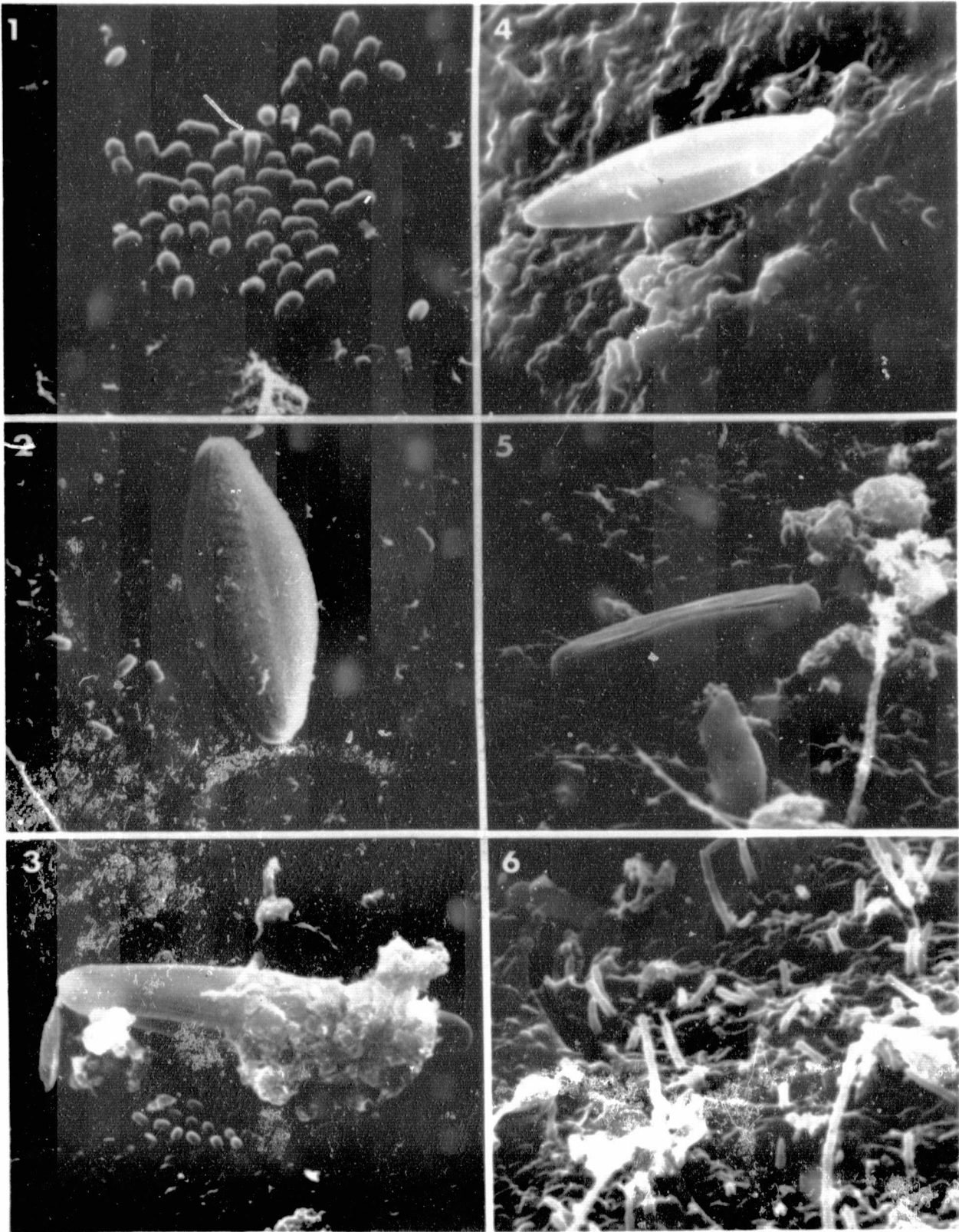
Figure 40. Parachute nylon (EH33-3) after exposure to microfouling for 24 hours, followed by 15 minute detergent treatment and tap and distilled water washing. Magnification 190X.

Figure 41. Nylon parachute material washed following 8 month exposure to microfouling in an aquarium. Fibrillar remains of organic matter not removed by washing can be seen. Magnification 86X.

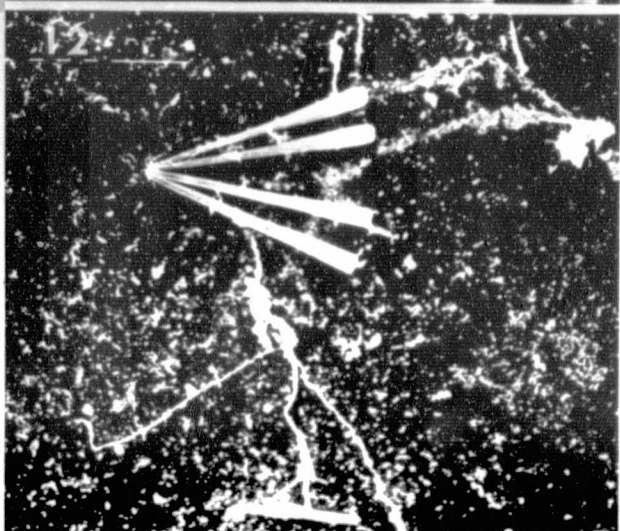
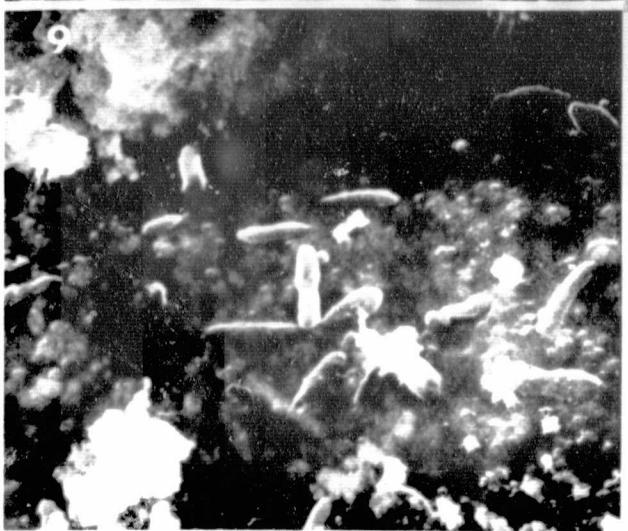
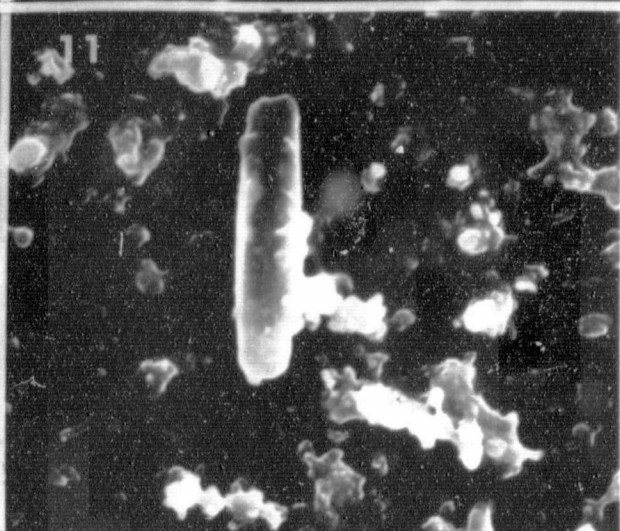
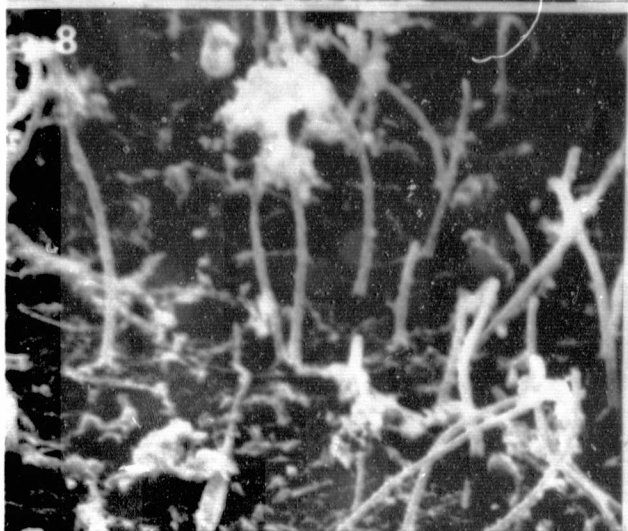
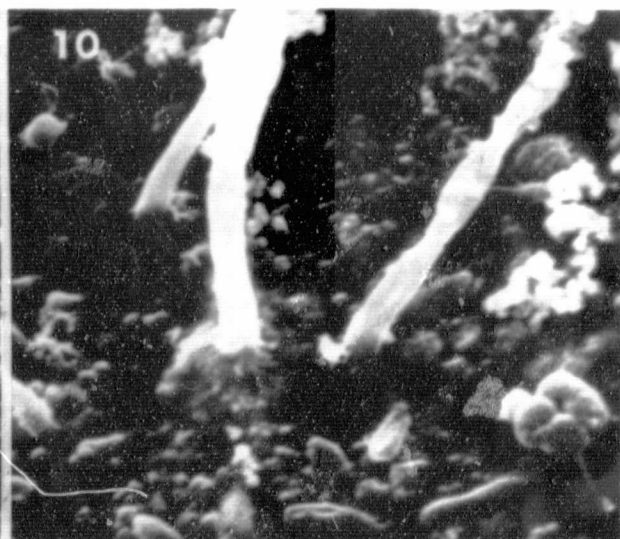
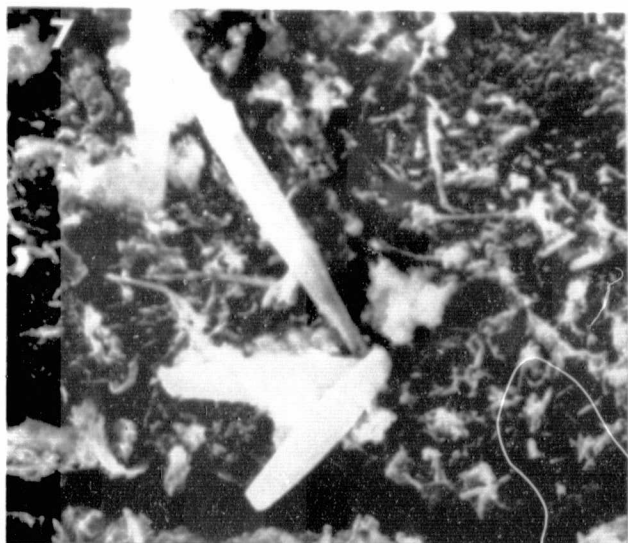
Figure 42. Nylon parachute material after exposure to microfouling for 8 months in an aquarium in the laboratory. (a) surface with heavy organic/inorganic deposition. Magnification 39X. (b) area in white box showing heavy deposition of material at junction of woven nylon. Magnification 195X.

Figure 43. Nylon parachute material washed after exposure for 8 months to microfouling in a laboratory aquarium. (a) surface, at 39X magnification, showing reduction in inorganic material. (b) area in white box, showing organic fibrillar material not removed by washing. Magnification 195X.

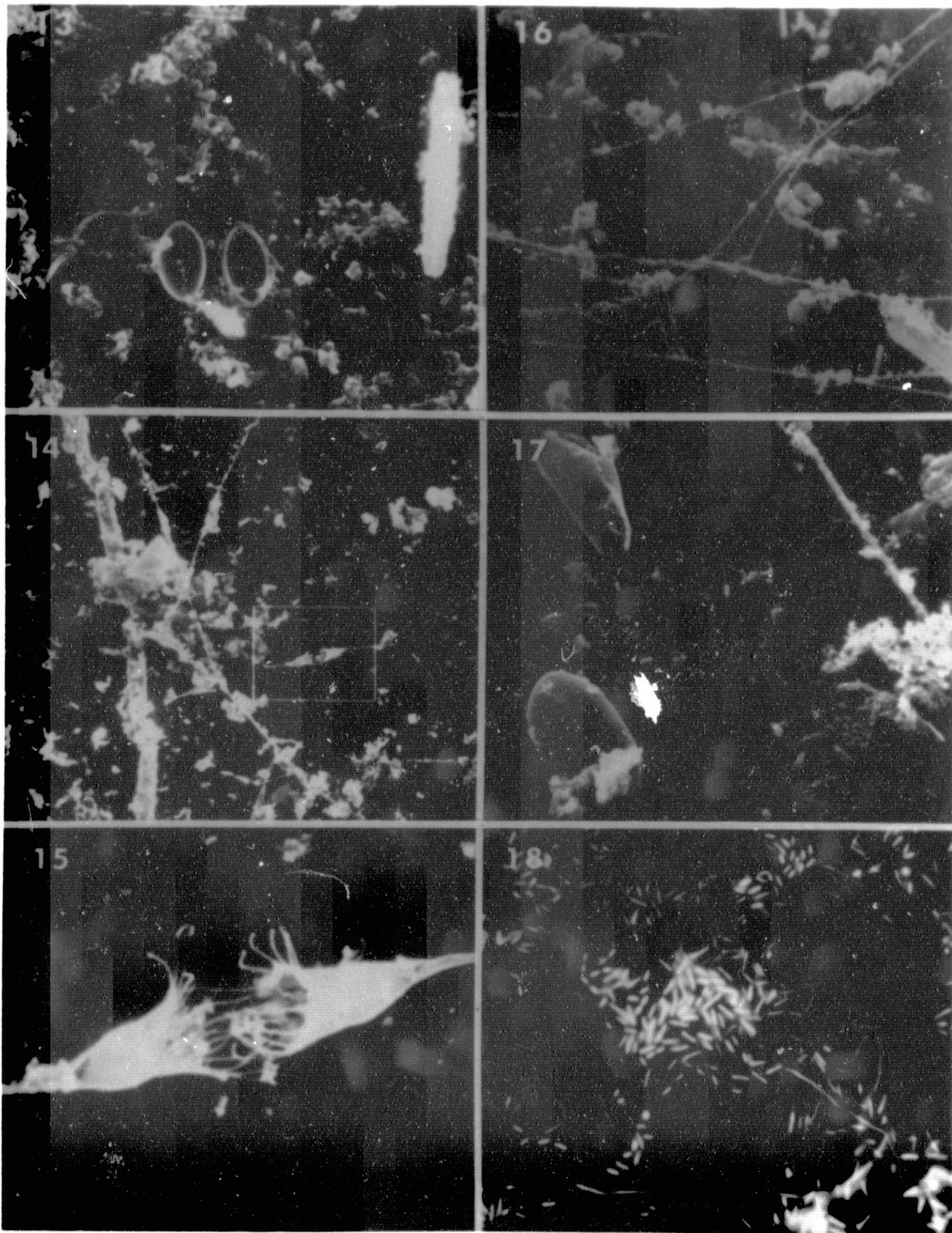
Figure 44. Change in mean number of attached bacteria on glass surfaces during a 4 hour exposure period in high concentration (●-●) and low concentration (○-○) flasks.



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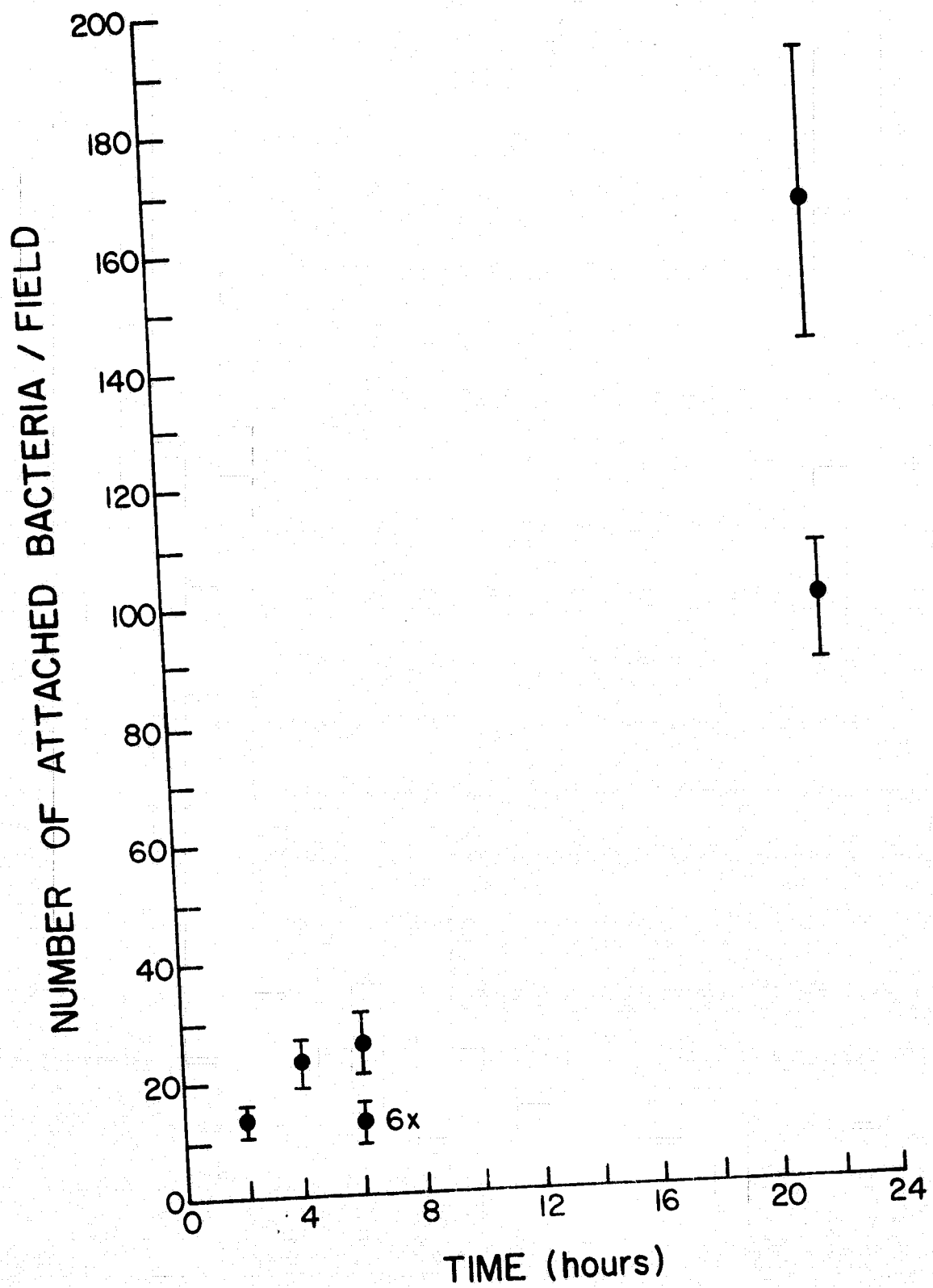
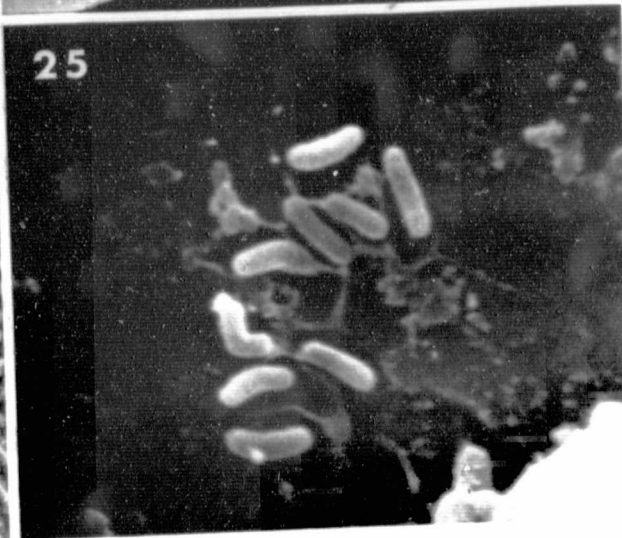
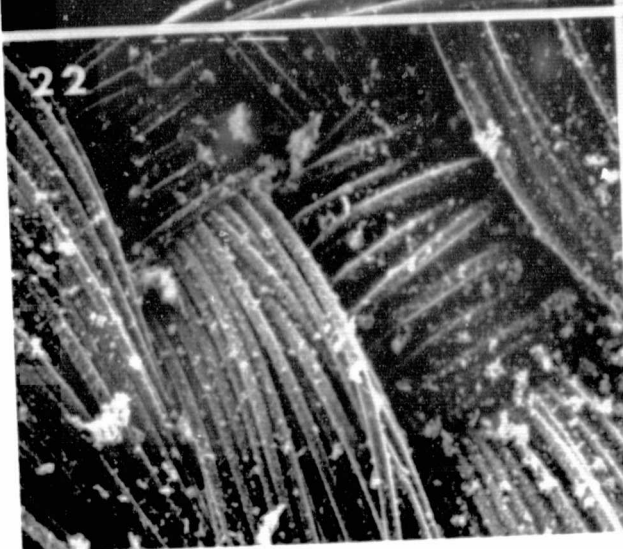
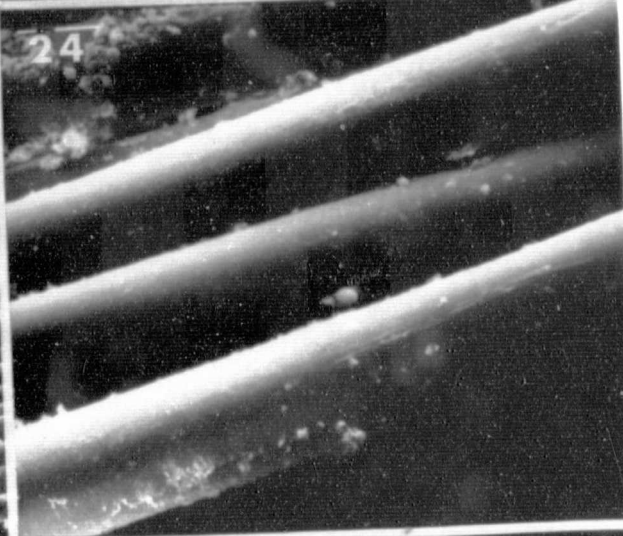
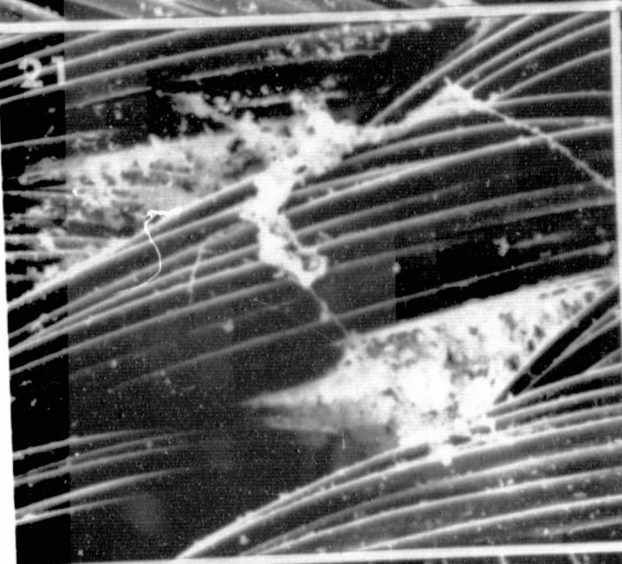
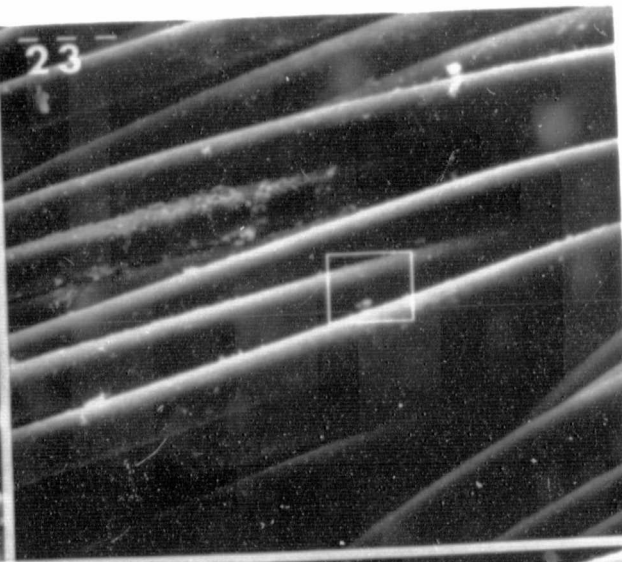
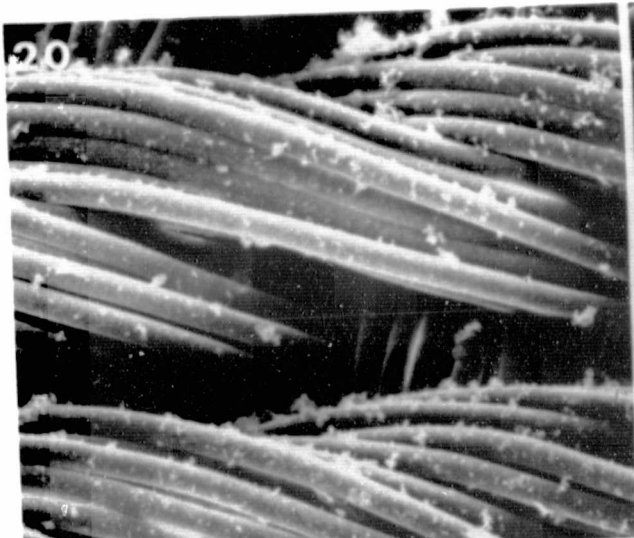
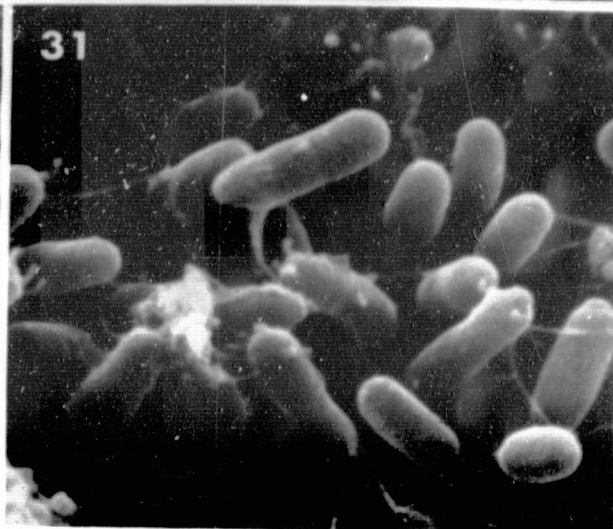
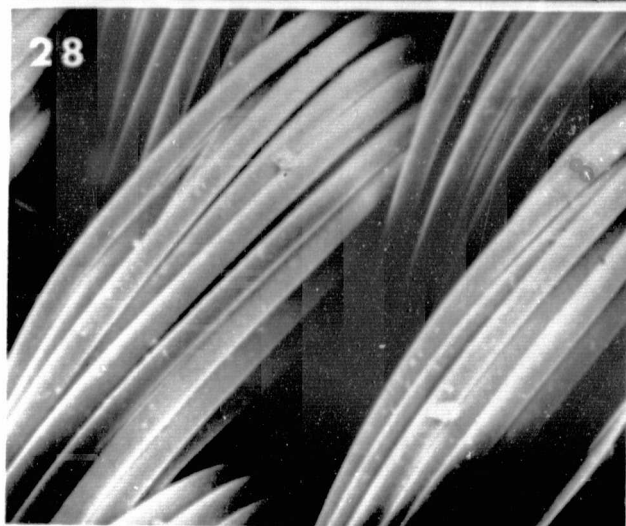
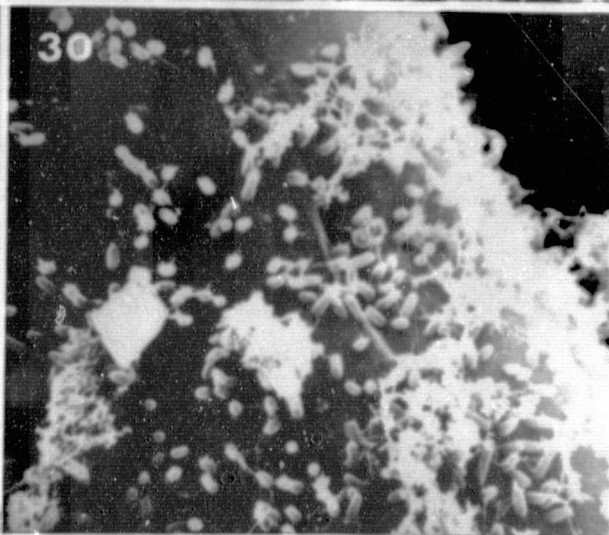
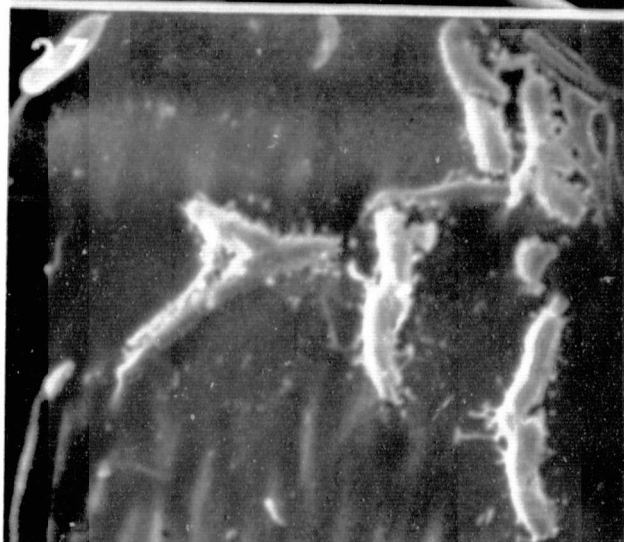
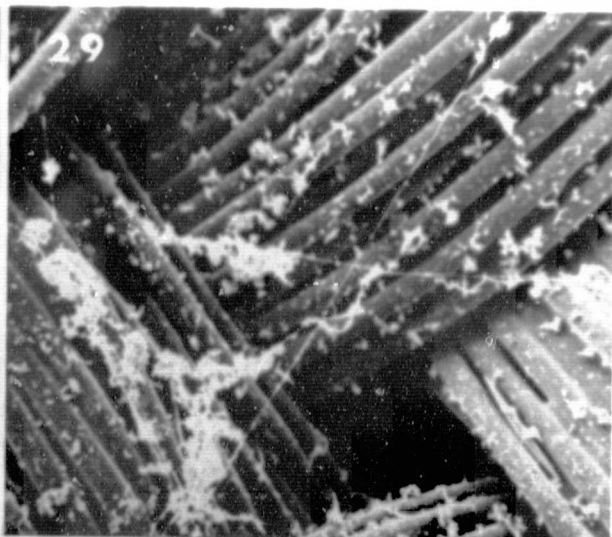
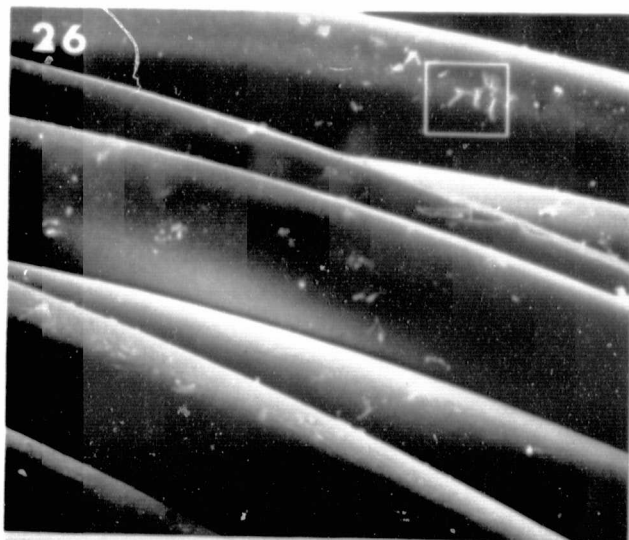


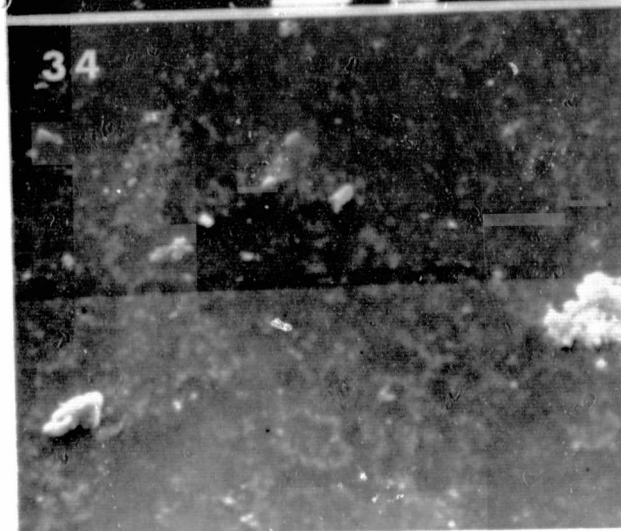
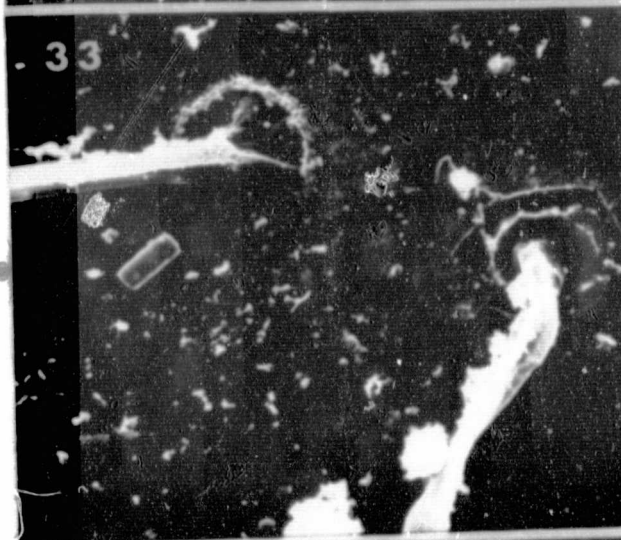
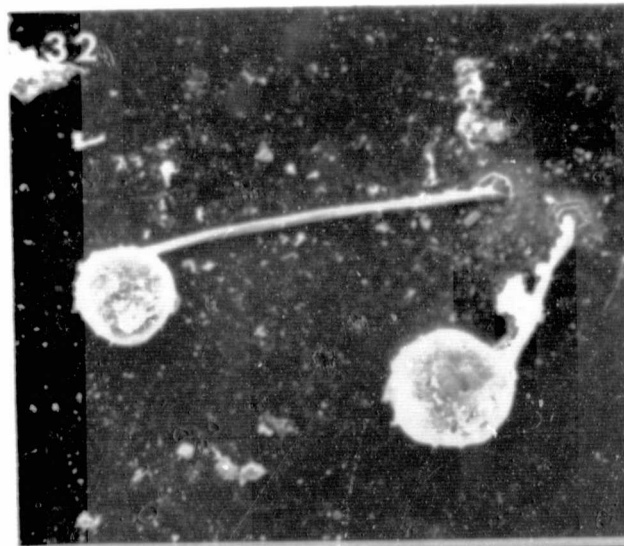
FIG. 19





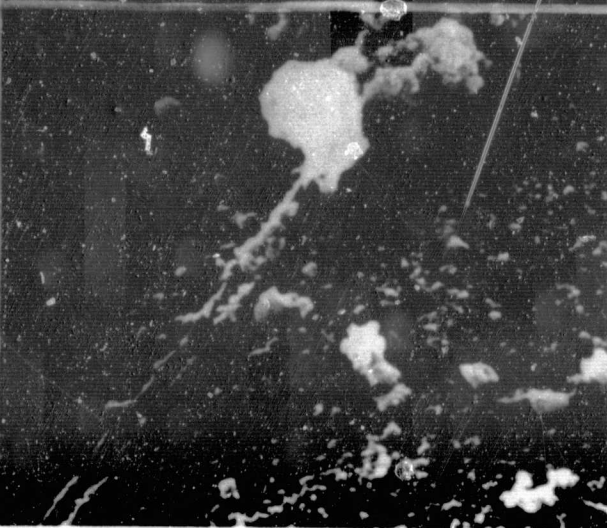
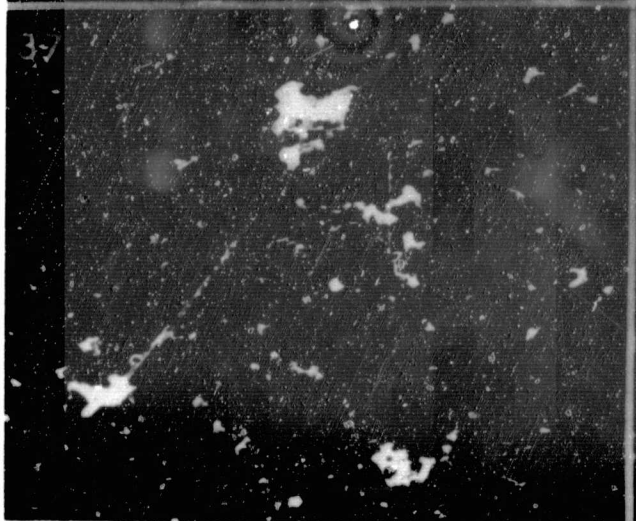
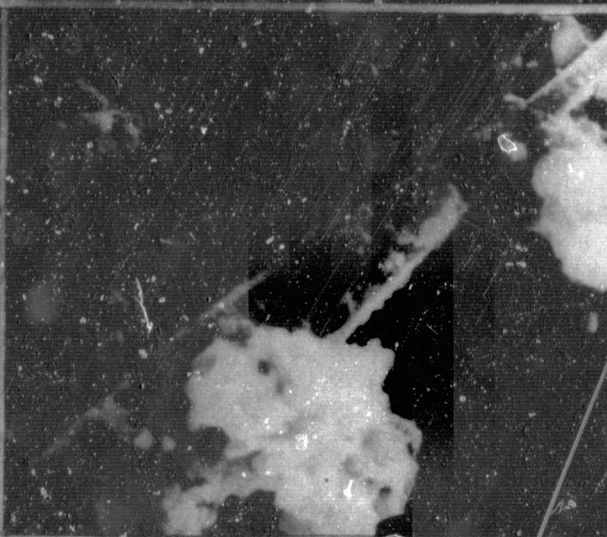
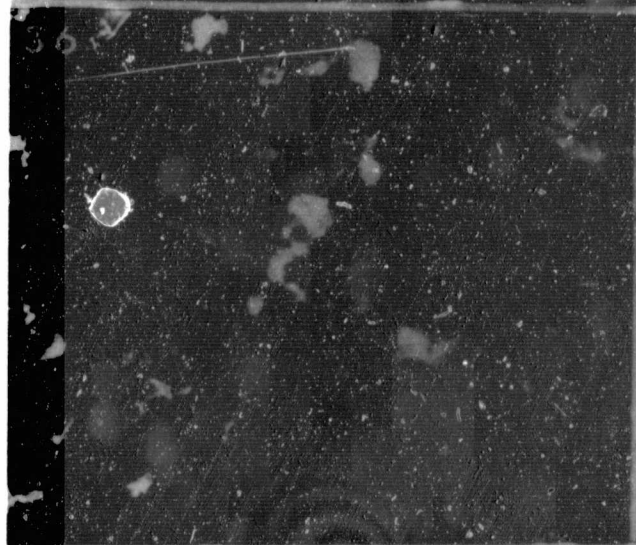
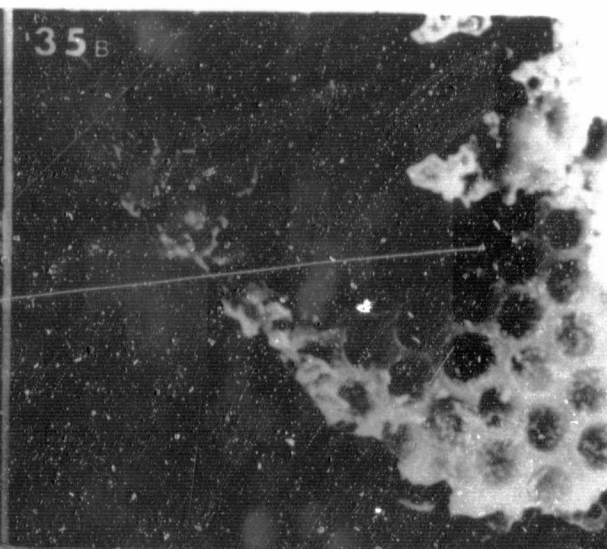
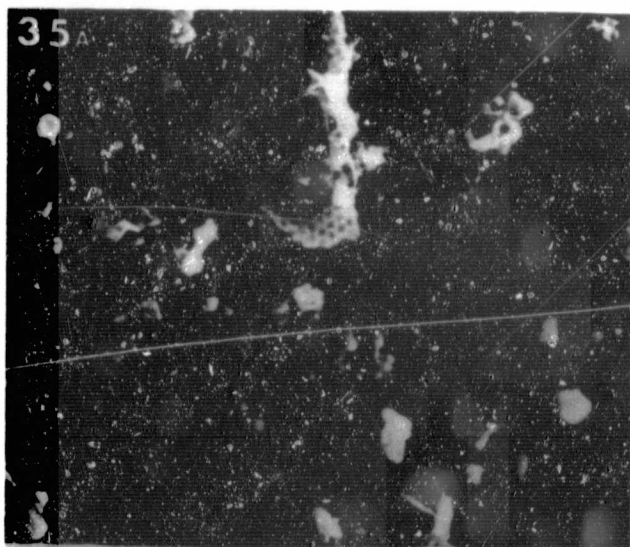
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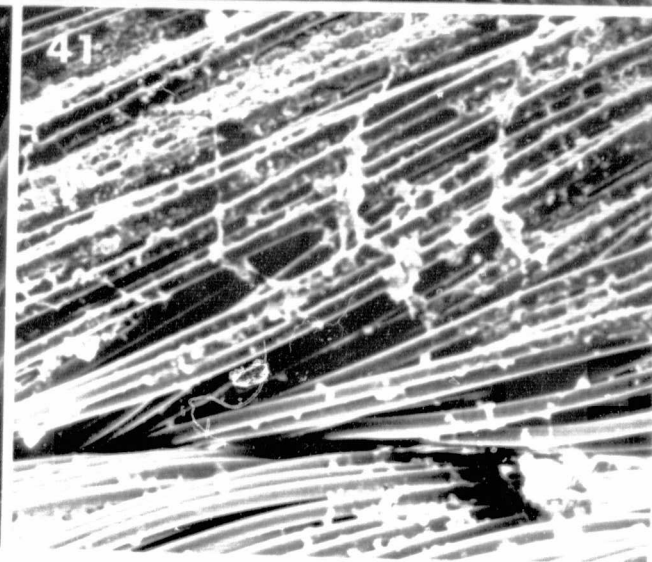
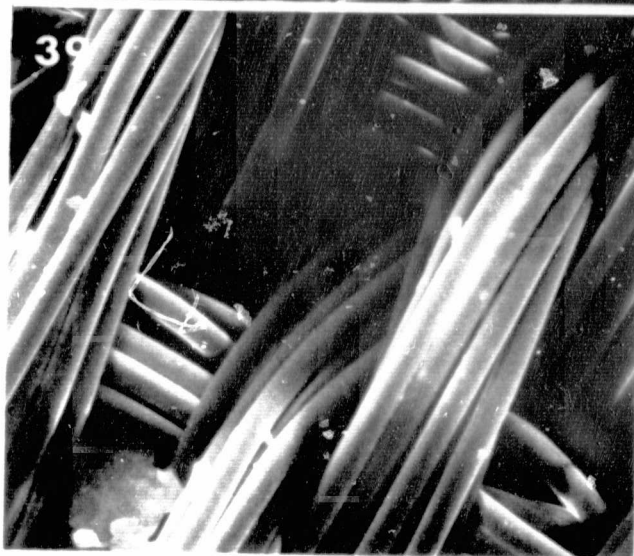
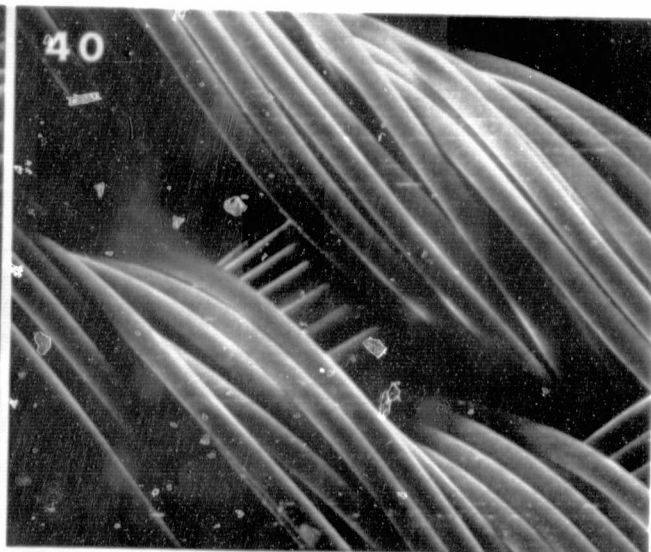




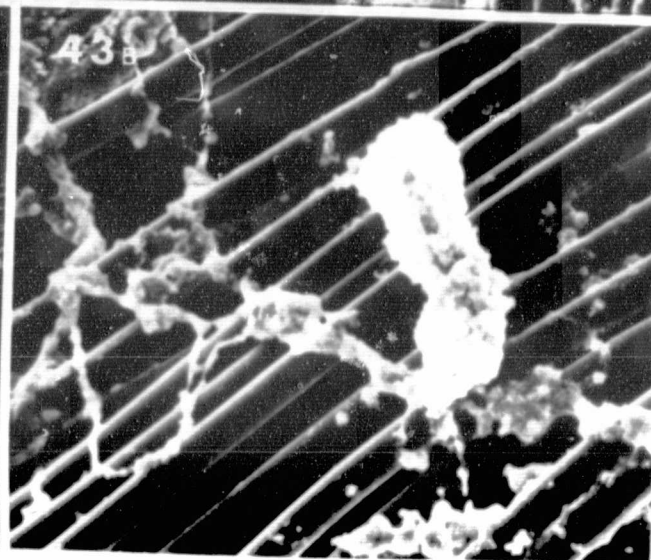
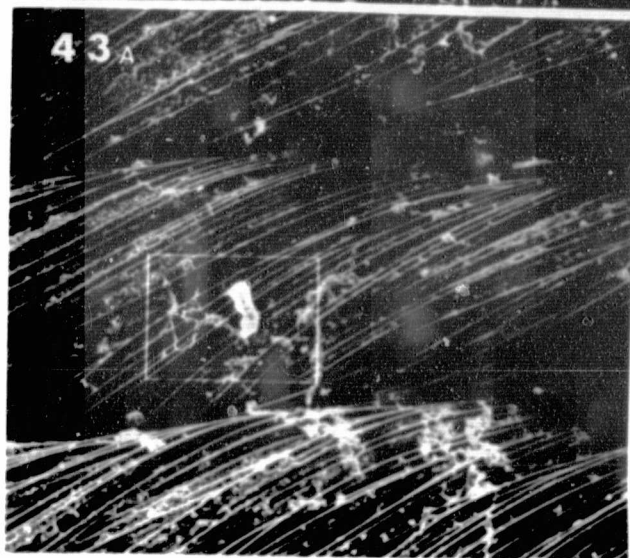
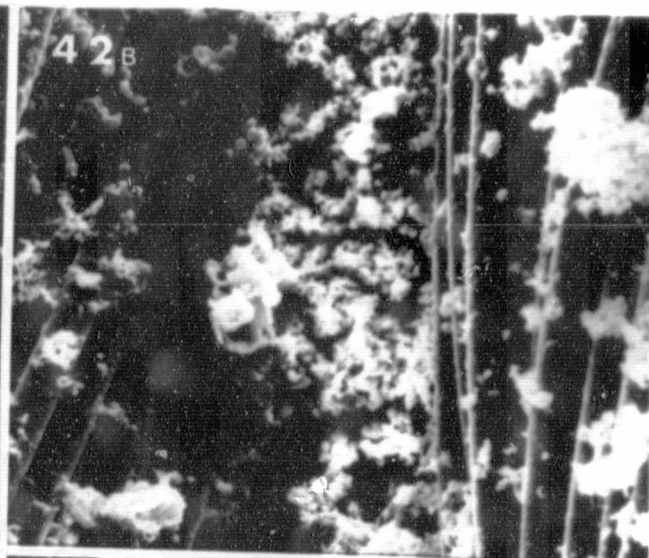
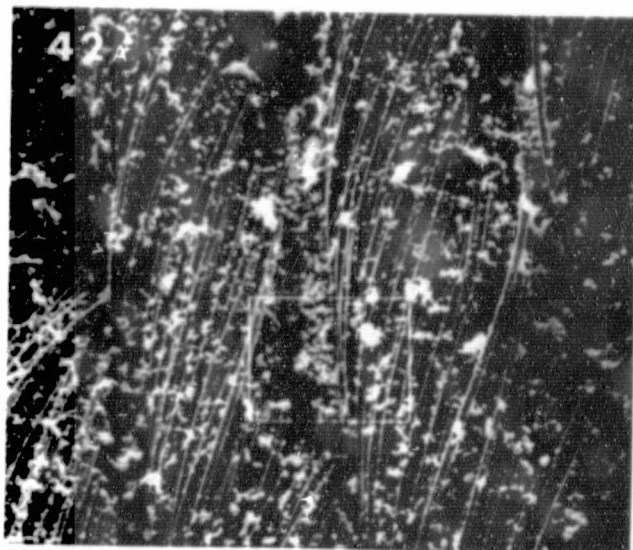
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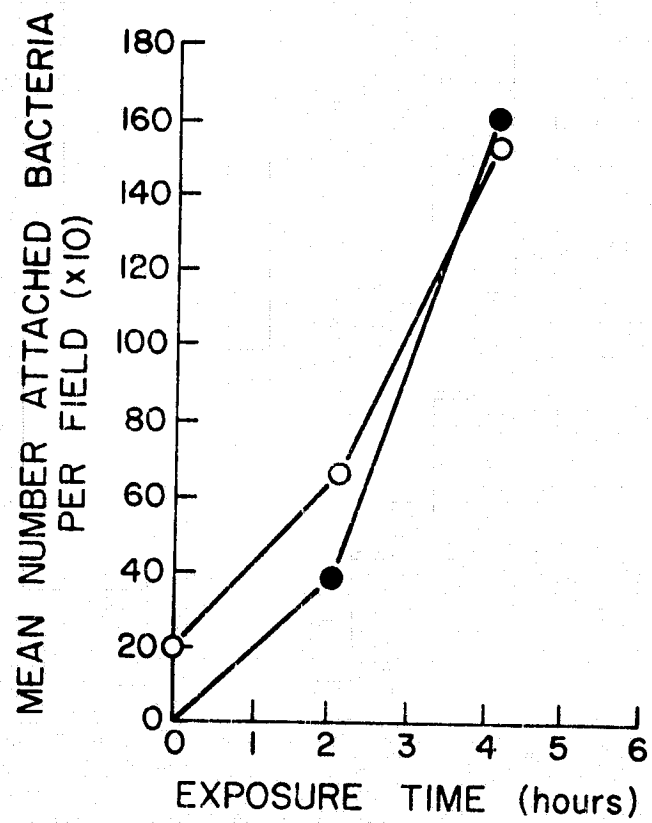


FIG. 44